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J Anim Sci 2000. 78:306-317.

Expression of two variants of growth hormone receptor messenger ribonucleic acid in porcine liver[1]

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ABSTRACT: Transcription of GH receptor (GHR) mRNA is initiated from multiple promoters. Most GHR mRNA arise from GHR Promoter 1 (GHR P1) and GHR P2, which transcribe GHR 1A and GHR 1B mRNA, respectively. Our objective was to characterize the expression of GHR 1A and GHR 1B mRNA in liver of neonatal intact (1 d of age) and castrated (14, 28, and 42 d of age) male pigs (Exp. 1; n = 6 per age group), intact male pigs treated with recombinant porcine ST (rpST) or control (Exp. 2; 21, 42, and 77 d of age; n = 4 pigs per treatment per age), and pregnant gilts treated with rpST (n = 6) or control (n = 7) (Exp. 3). Tissue samples were collected at slaughter for mRNA analyses. The porcine GHR 1A and GHR 1B cDNA were cloned and were homologous to GHR cDNA isolated from other species. Ribonuclease protection assays were used to measure GHR 1A and GHR 1B mRNA. Liver expressed GHR 1A and GHR 1B mRNA, whereas muscle, uterus, and ovary expressed GHR 1B mRNA. The GHR 1A mRNA in the liver of neonatal intact and castrated male pigs (Exp. 1) was expressed at very low levels on d 1, 14, and 28, and two of six pigs expressed a high level of GHR 1A on d 42. The GHR 1B mRNA, however, was detected at all ages (d 1 through 42), and the amount of GHR 1B increased ($P < .05$) on d 42. The liver of intact male pigs (Exp. 2) expressed GHR 1B mRNA by 21 d, whereas a high level of GHR 1A mRNA was not detected until d 42 ($P < .10$). Administration of rpST had no effect on expression of GHR 1A or GHR 1B mRNA in pigs younger than 77 d (Exp. 2), but it tended to decrease ($P < .10$) GHR 1A mRNA but not GHR 1B mRNA in pregnant gilts (Exp. 3). In conclusion, GHR mRNA in porcine liver was composed of at least two variants (GHR 1A and GHR 1B). The GHR 1B mRNA was the major GHR mRNA in pig liver before 77 d of age. The GHR 1A mRNA increased after 42 d of age and tended to undergo specific down-regulation in response to rpST in pregnant gilts.

Key Words: Pigs, Somatotropin, Hormone Receptors, Development

 J. Anim. Sci. 2000. 78:306–317

Introduction

Variants of GH receptor (**GHR**) mRNA exist in the liver of humans (Pekhletsky et al., 1992), cattle (Heap et al., 1996), sheep (Adams, 1995), rats (Baumbach and Bingham, 1995), and mice (Southard et al., 1995), but they have not been characterized in pigs. Most of the GHR mRNA variants arise from alternative splicing within exon 1 of the GHR. The presence of alternative exon 1 suggests that several promoters control GHR mRNA transcription (Godowski et al., 1989). Initiation of transcription from multiple promoters may provide a mechanism for developmental and tissue-specific GHR expression. Most GHR mRNA arise from GHR Promoter 1 (**GHR P1**) and GHR P2, which transcribe GHR 1A and GHR 1B mRNA, respectively. The tissue-specific activity of each promoter is different because GHR P1 is active in liver, whereas GHR P2 is active in liver as well as other tissues (Schwartzbauer and Menon, 1998).

The total liver GHR mRNA increased but total muscle GHR mRNA did not change in pigs from birth to one yr of age (Schnoebelen-Combes et al., 1996). The tissue-specific pattern of GHR expression in growing pigs suggested that GHR gene expression was controlled by different mechanisms in liver and muscle that may involve different GHR promoters. The analyses of total GHR mRNA in the preceding study were done by using a GHR cDNA probe for the coding region of the

[1]This work was supported by a grant from the National Research Initiative Competitive Grants Program. Grant number USDA CSREES 95-37205-2312 was awarded to M. C. Lucy. Contribution from the Missouri Agric. Exp. Sta.; Journal Series Number 12,849. Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable. The authors are grateful to Y. Kobayashi and H. Jiang for technical assistance and C. K. Boyd for preparation of figures.

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Received March 11, 1999.
Accepted June 23, 1999.

GHR. The cDNA probe could not differentiate between the unique GHR variants, and, hence, the unique activity of the GHR P1 and GHR P2 in postnatal pigs was not determined. Our objectives for the present study, therefore, were to isolate and sequence the cDNA for the porcine GHR 1A and GHR 1B mRNA, develop ribonuclease protection assays (**RPA**) for measuring porcine GHR 1A and GHR 1B mRNA, and determine the tissue-specific and developmental regulation of GHR 1A and GHR 1B mRNA within postnatal liver. The effect of exogenous GH on the expression of GHR 1A and GHR 1B mRNA was also tested.

Materials and Methods

cDNA Cloning and Sequencing of GHR Variants and Glyceraldehyde 3-Phosphate Dehydrogenase

The GHR 1A and GHR 1B cDNA were cloned by reverse transcription and PCR (**RT-PCR**). Briefly, 1 μg of total RNA from adult porcine liver was incubated with 24 units of RNasin (Promega, Madison, WI), 6.25 m*M* dATP, 6.25 m*M* dCTP, 6.25 m*M* dGTP, 6.25 m*M* dTTP, 34 p*M* reverse primer (5′-GTGCAGTTCATACT CCAG-3′), 5-strength buffer, and 15 units of avian myeloblastosis virus reverse transcriptase (Promega) for 1 h at 42°C. The reverse primer (located within GHR exon 6) was originally used for bovine cloning and had one nucleotide difference from the porcine sequence (Cioffi et al., 1990). After the initial incubation, 5 μL of the reverse transcription solution was added to a Ready-To-Go PCR bead (Pharmacia Biotech, Piscataway, NJ) with 10 pmol forward and reverse PCR primers in a final volume of 25 μL. The mixture was vortexed and overlaid with two drops of mineral oil prior to amplification. Thermal cycle program was 1 cycle of 94°C for 4 min, 35 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 2 min, and a final extension at 72°C for 7 min. The forward primer used for cloning GHR 1A (5′-GCCATA AAGCCTGGAGGAA-3′) was based on sequence within exon 1A of bovine (Hauser et al., 1990) and ovine (Adams et al., 1990; Figure 1A). The forward primer for cloning GHR 1B (5′-ACGCGAACCGCGCTCTCTCTCC-3′) was based on exon 1B of bovine (Heap et al., 1996; Figure 1B). The reverse primer for cloning GHR 1A (5′-GTGCAGTTCATACTCCAG-3′) was located within GHR exon 6, and the reverse primer for cloning GHR 1B (5′-TCTGCAGACTCTGAGATGCT-3′) was located within GHR exon 3. A 660-bp cDNA for GHR 1A and a 162-bp cDNA for GHR 1B were subcloned into the pGEM T-Easy vector (Promega). The GHR 1A plasmid was then digested with Pst I (restriction enzyme site within exon 3) and relegated to create a smaller insert. The final GHR 1A clone contained 184 bp of GHR 1A cDNA. Two individual clones were selected for GHR 1A and GHR 1B, respectively, and the cDNA sequences were determined by automated DNA sequencing. Homologies of porcine GHR 1A and GHR 1B cDNA with GHR cDNA isolated from other species were determined by using the GeneStream alignment tool (Institute of Human Genetics, Montpellier, France).

The cDNA for porcine glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**; control) was amplified by RT-PCR and subcloned for the production of ribonucleotide probes. The forward primer (5′-ACACTGAGGACCAG GTTG-3′) and reverse primer (5′-TGGTCGTTGAGGG CAATG-3′) for cloning porcine GAPDH were based on the porcine GAPDH (GenBank: U82261). A 91-bp cDNA for GAPDH was subcloned into the pGEM T-Easy (Promega) and then recloned into pGEM 3Z vector (Promega). The identity and orientation of cDNA were confirmed by automated DNA sequencing.

Animals and Treatments

Experiment 1. Neonatal (1 d of age) and castrated male pigs were used to study the developmental expression of GHR 1A and GHR 1B mRNA in liver. Details of animal procedures have been described previously (Matteri and Carroll, 1997). In brief, crossbred male pigs were assigned by litter and body weight to be slaughtered at 1 d of age or castrated on 3 d of age and slaughtered at 14, 28, or 42 d of age (n = 6 per age group). Weaning was at 21 d of age. Pigs were killed by electrocution followed by exsanguination. Following slaughter, liver samples (approximately 1 g) were placed on dry ice until being stored at –80°C.

Experiment 2. Intact boars were used to study the developmental pattern and GH-dependent expression of GHR 1A and GHR 1B mRNA in liver. The details of animal procedures were described previously (Carroll et al., 1998). Crossbred boars were allotted to one of three age groups (7, 28, or 63 d). Within each age group, four pigs were randomly assigned to receive recombinant porcine ST (**rpST**; Monsanto Co., St. Louis, MO; daily i.m. injection of 200 μg/kg BW), and four pigs were randomly assigned to the control group (daily i.m. injection of vehicle). Injections began on d 7, 28, or 63 and were given for 14 d. Pigs were killed by electrocution followed by exsanguination at 21, 42, and 77 d of age (approximately 24 h after the last injection). Liver was collected at slaughter, placed on dry ice, and stored at –80°C. Longissimus muscle samples were also collected but were not analyzed in the present study except for a preliminary test of GHR 1A and GHR 1B tissue specificity (three 77-d-old control pigs).

Experiment 3. Crossbred gilts were used to determine the effect of rpST on the expression of GHR 1A and GHR 1B mRNA during pregnancy (Sterle et al., 1998). Beginning on d 30 of pregnancy, six gilts received daily i.m. injections of 5 mg of rpST, and seven gilts received 1 mL of saline (control) for 14 d. On d 44 of pregnancy, gilts were slaughtered by electrocution and exsanguination (approximately 24 h after the last injection). Liver samples (approximately 5 g) were frozen in liquid nitrogen and stored at –80°C. Uterus and ovary samples were also collected but were not analyzed in the present

study except for a preliminary test of GHR 1A and GHR 1B tissue specificity (one control pig).

RNA Preparation and Ribonuclease Protection Assay (RPA)

Total cellular RNA was isolated by using the TRIZOL procedure (GibcoBRL, Gaithersburg, MD) and dissolved in water. The concentration and purity were determined by calculating the ratio of absorbances at 260 and 280 nm. A sample of RNA (2.5 μg) was electrophoresed in a 1% TBE agarose gel and stained with ethidium bromide to verify integrity and quantity. Cellular RNA samples that had intact 28S and 18S ribosomal bands were used in subsequent analyses. Isolated RNA was stored at –80°C until RPA.

Plasmids containing GHR 1A, GHR 1B, IGF-I (Sterle et al., 1998), and GAPDH were linearized by digestion with *Eco*RI, *Sal*I, *Xba*I, and *Xba*I, respectively. The digested plasmids were extracted with phenol/chloroform and ethanol precipitated. Antisense ribonucleotide probes were generated by using a RNA transcription kit (Stratagene, La Jolla, CA). Linearized plasmids (approximately 200 ng) were incubated with SP6 (GHR 1A and IGF-I) or T7 (GHR 1B and GAPDH) RNA polymerase (Promega), [^{32}P]rCTP (New England Nuclear, Boston, MA), appropriate buffers, and nucleotides to yield ribonucleotide probes that were antisense to the specific mRNA. The GHR 1A riboprobe was generated from a 184-bp cDNA that contained GHR 1A exon 1 (63 bp) as well as GHR exon 2–3 (121 bp). The GHR 1B riboprobe was generated from a 162-bp cDNA that contained GHR 1B exon 1 (39 bp) as well as GHR exon 2–3 (123 bp). The riboprobes for IGF-I and GAPDH were generated from 472-bp (Sterle et al., 1998) and 91-bp cDNA, respectively. The size of the riboprobes was verified by comparison to a radiolabeled 100-bp ladder.

Ribonuclease protection assays were performed with 25 μg of total cellular RNA by using the RPA II kit (Ambion Inc., Austin, TX). The initial RPA included GHR 1A, IGF-I, and GAPDH riboprobes. The GHR 1B riboprobe was used in a later RPA to confirm the expression of GHR 1B mRNA. Protected mRNA were identified by their electrophoretic mobility through a 6% acrylamide, 8 *M* urea gel (Acryl-A-Mix-6, Promega). Gels were dried, and autoradiography was performed by using XOMAT-AR film (Eastman Kodak, Rochester, NY) at –80°C with intensifying screens. The probe length and protected fragment length were verified with [^{32}P]DNA standards. Each RPA contained a negative control (yeast tRNA) and two or three positive control (adult pregnant porcine liver RNA) samples. The intensity of protected fragments was quantified by using GP Tools (BioPhotonics Corp., Ann Arbor, MI).

Statistical Analysis

The GAPDH signal was designed as an internal control for correction of mRNA data. We found, however, that there was an effect of age ($P < .05$) on GAPDH for Exp. 1. The greatest GAPDH expression was observed in samples collected from 1-d-old pigs. Therefore, data from Exp. 1 were not adjusted for GAPDH. There was no effect of age for GAPDH in Exp. 2 (1-d-old pigs were not studied in Exp. 2). The mRNA data from Exp. 2, therefore, were normalized to the GAPDH signal. The analyses for Exp. 3 were not normalized because the RPA were done before the GAPDH was cloned. Heterogeneous variances for the mRNA data were encountered for the GHR 1A and GHR 1B RPA in Exp. 1 and the GHR 1A RPA in Exp. 2. Therefore, the data were $\log_{10}$ transformed before statistical analyses. In each case, $\log_{10}$ transformation corrected the heterogeneous variance. Heterogeneous variance was not encountered for the GHR 1B RPA in Exp. 2, and the data were not $\log_{10}$ transformed. Data were analyzed with least squares analyses of variance using the GLM Procedure of SAS (1985). Two RPA were done for each of Exp. 1 and 2 because only 12 test samples could be included in each RPA. Therefore, the effect of assay was included in the statistical analyses. The statistical model for Exp. 1 included the main effects of age and assay and the age × assay interaction. The effects of age were tested by using age × assay as the error term. Experiment 2 was analyzed as a 2 × 3 factorial with the main effects of treatment (rpST), age, assay, and their interactions. The main effects of treatment, age, and treatment × age were tested by using age × assay, treatment × assay, and age × treatment × assay, respectively, as the error terms. Means were separated with Duncan's multiple range test. The statistical model for Exp. 3 included the effect of treatment. Data are presented as means ± SEM for Exp. 1 and 2 (the true means and not the log-transformed means are reported) or least squares means ± SEM for Exp. 3. Data were considered significant at $P < .05$ unless otherwise indicated.

Results

cDNA Sequence of GHR 1A and GHR 1B

Porcine cDNA for GHR 1A and GHR 1B were cloned and sequenced. The clones were not designed to be full-length cDNA. Instead, partial cDNA, diagnostic for GHR 1A and GHR 1B mRNA, were isolated. The exon-1 sequence of GHR 1B shared little homology with GHR 1A (25%). The homologies of porcine GHR 1A to the analogous human (Pekhletsky et al., 1992), bovine (Hauser et al., 1990), ovine (O'Mahoney et al., 1994) and rabbit (Leung et al., 1987) sequences were 88.2, 83.8, 86.8, and 72.2%, respectively (Figure 1A). There was little homology for porcine GHR 1A with mouse or rat GHR (data not shown). The homologies of porcine GHR 1B with the analogous human (Pekhletsky et al., (1992), bovine (Heap et al., 1996), ovine (Adams, 1995), rat (Domene et al., 1995), and mouse (Southard et al., 1995) sequences were 69.2, 87.5, 87.2, 64.1, and 69.2%, respectively (Figure 1B).

A

GHR 1A

	-70	-60	-50	-40	-30	-20	-12
hV1	GATATAAAGC	CTGGAGGAAA	C-A-A--TACGAAA	ATCCAGCCTC	TATTTCAGCA	ATATCTGCCG	GACTATTG
bov1A	GCCATAAAGC	CTGGAGGAA-	CCA----TACGAAA	ATCCAGCCTC	TGTTTCAGGA	GTGTCTGCTG	GACTCTTG
ov1A	GCCATAAAGC	CTGGAGGAAA	CCA----TACGAAA	ATCCAGCCTC	TGTTTCAGGA	TTGTCTGCCG	GACTATTG
rab	GATACAAAGC	CTGGAGGGAG	CTACACGTTCAAAA	ATCCAACTTC	TATATCAGGA	ACATCTGCTG	GACTATTG
p1A	GCCATAAAGC	CTGGAGGAAA	CCA----TACAAAA	ATCCAGCCTC	TATCT-AG-A	ATATCTGCCG	GACTTTTG

Forward primer

B

GHR 1B

	-40	-30	-20	-12
hV2	ACGGAACCCGC	GCTCTCTGAT	CAGAGGCGAA	GCTCGGAG
bov1B	ACGCGAACCGC	GCTCTCTCTC	CCGAGGCTCG	GCTCGCAG
ov1B	ACGCGAACCGC	GCTCTCTCTC	CCGAGGCGAG	GCTCGGAG
rv1	ACCGGAACCCG	GCTCTGTCTC	CCAAGGCGAA	CGTTCGAG
mL2	ACGA-ACCCGC	GTTCTGTCTC	CCGAGGCGAA	ACTCCGAG
p1B	ACGCGAACCGC	GCTCTCTCTC	CGGAGGCTCG	GTTCAGAG

Forward primer

Figure 1. Alignment of exon 1 sequences of GH receptor (GHR) 1A (panel A) and GHR 1B (panel B) from human 1 (hV1) and 2 (hV2; Pekhletsky et al., 1992), bovine 1A (bov1A; Hauser et al., 1990) and 1B (bov1B; Heap et al., 1996), ovine 1A (ov1A; O'Mahoney et al., 1994) and 1B (ov1B; Adams, 1995), rabbit (rab; Leung et al., 1987), rat V1 (Domene et al., 1995), mouse L2 (Southard et al., 1995) and porcine 1A (p1A) and 1B (p1B) cDNA. Dashes show deletions. The forward primers used to clone GHR 1A and GHR 1B are underlined. Sequences extended to position –12 (end of exon 1) relative to the ATG start codon. Numbering follows the human cDNA.

Tissue-Specific Expression of GHR 1A and GHR 1B mRNA

Preliminary tests were done to determine the tissue-specificity of GHR 1A and GHR 1B mRNA. The two riboprobes (GHR 1A and GHR 1B) were derived from two alternative GHR with different exon 1 but identical exon 2–3. The GHR 1A RPA yielded a 184-bp protected fragment that corresponded to GHR 1A mRNA and a 121-bp exon 2–3 fragment that corresponded to GHR variant(s) other than GHR 1A. The exon 2–3 fragment was present because GHR with nonhomologous exon 1 will hybridize with exon 2–3 of the probe and produce a signal within the RPA. The GHR 1B RPA yielded a 162-bp protected fragment that corresponded to GHR 1B mRNA and a 123-bp exon 2–3 fragment that corresponded to GHR variant(s) other than GHR 1B. The GHR 1A mRNA was detected in liver but not muscle of 77-d-old pigs (Figure 2A; one of three pigs expressed a high level of GHR 1A, and two of three had low levels that required prolonged exposure). In contrast, the GHR 1B mRNA was detected in both liver and muscle, although the signal in liver was considerably greater than in muscle. An exon 2–3 fragment was not detected in muscle of the GHR 1B RPA. This suggested that GHR mRNA in muscle is primarily GHR 1B mRNA.

The RNA isolated from the liver, uterus, and ovary of a pregnant gilt was analyzed using the GHR 1A and GHR 1B RPA (Figure 2B). Liver expressed GHR 1A and GHR 1B mRNA. Uterus and ovary, however, expressed GHR 1B but did not express GHR 1A mRNA. Expression of GHR 1B in uterus and ovary was considerably less than liver. An exon 2–3 fragment was not detected for uterus or ovary when each tissue was tested with the GHR 1B RPA. This suggested that GHR mRNA in uterus and ovary is primarily GHR 1B mRNA.

Developmental and Hormonal Regulation of GHR 1A and GHR 1B mRNA

Experiment 1. The GHR 1A mRNA was expressed at low levels at 1, 14, or 28 d with a signal near background

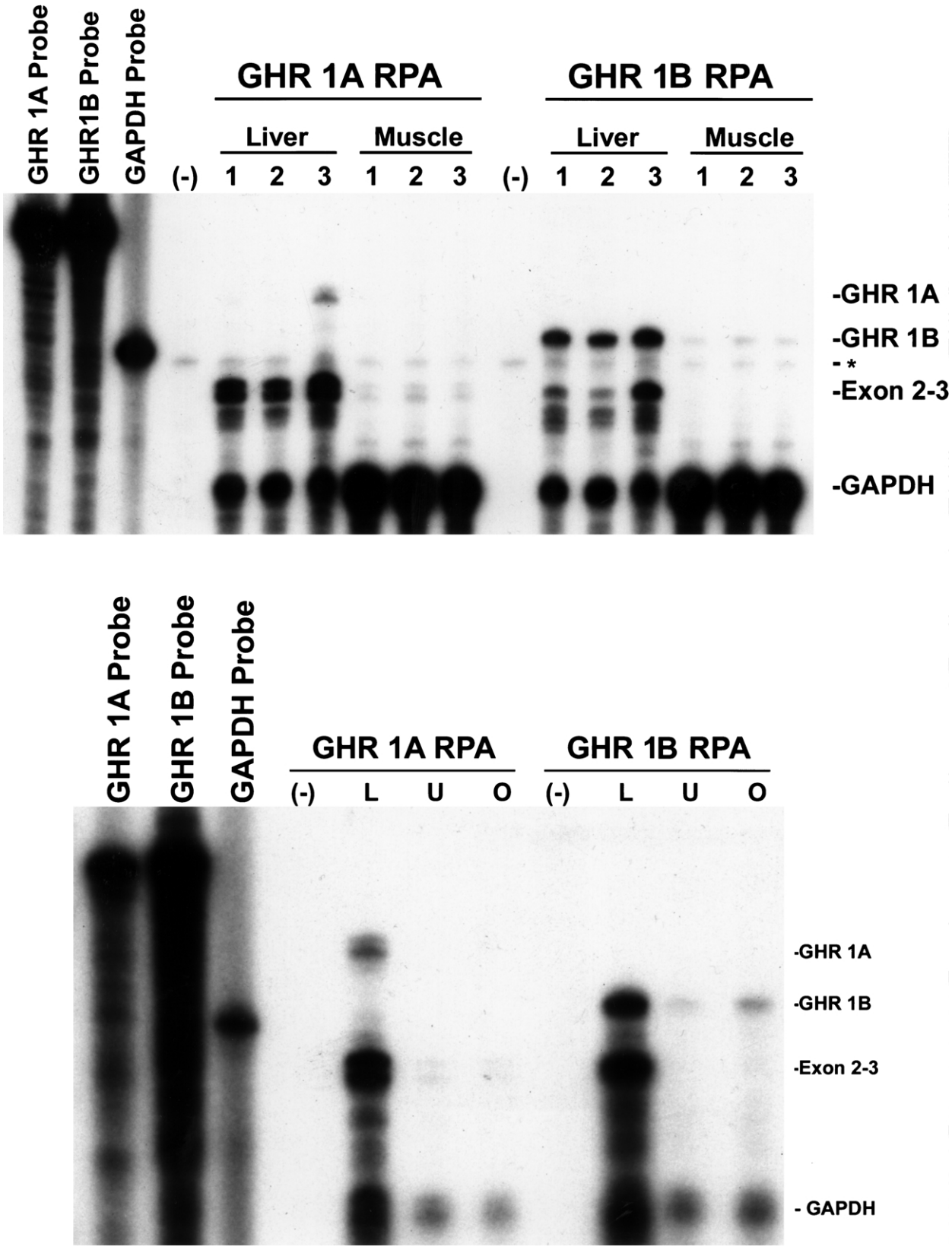

Figure 2. (A) Autoradiograph of a ribonuclease protection assay for GH receptor (GHR) 1A, GHR 1B, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA in liver and longissimus muscle in each of three 77-d-old male pigs. (B) Autoradiograph of a ribonuclease protection assay for GHR 1A, GHR 1B, and GAPDH mRNA in liver (L), uterus (U), and ovary (O) of a pregnant pig. For each ribonuclease protection assay, locations of GHR 1A, GHR 1B, GHR exon 2–3, and GAPDH protected fragments are shown at right. Undigested probes (left) as well as negative control (yeast RNA) (–) are also shown; the asterisk denotes residual of undigested GAPDH probe.

(i.e., the protected fragment could not be seen after short exposure [Figure 3A] but could be seen after prolonged exposure when a haze from nonspecific radioactive signal was also visible). Liver IGF-I mRNA was present at all ages as well, but the signal was also near background. At 42 d, two of six pigs expressed readily detectable GHR 1A and IGF-I, but statistical analyses were not performed on data for the two pigs because of small sample size. For most pigs in Exp. 1, GHR 1A was expressed at very low levels. Therefore, the exon 2–3 fragment in the GHR 1A assay represented total GHR. There was a tendency for an effect of age ($P < .10$) on the exon 2–3 signal (total GHR mRNA). The exon 2–3 signal increased from d 1 to d 14, decreased on d 28, and then increased to its highest levels on d 42 (Figure 3C).

The GHR 1B mRNA and other unidentified GHR variant(s) (the exon 2–3 signal in the GHR 1B assay) were detected at all ages (Figure 3B). There was an effect of age on the GHR 1B signal ($P < .05$) and the exon 2–3 signal ($P < .05$) in the GHR 1B assay (Figure 3C). The pattern of expression for GHR 1B and exon 2–3 in the GHR 1B assay was similar to exon 2–3 in the GHR 1A assay (increased from d 1 to 14, decreased on d 28, and then increased on d 42).

Experiment 2. The GHR 1A, exon 2–3 (within the GHR 1A assay), and IGF-I mRNA were expressed by 21 d of age (Figure 4A; the expression of GHR 1A on d 21 could only be detected after prolonged exposure times). There was no effect of rpST treatment or treatment × age interaction for the expression of GHR 1A, exon 2–3, or IGF-I mRNA. Considerable variation for the expression of GHR 1A mRNA existed because some pigs intensely expressed GHR 1A by 42 d, whereas other pigs expressed very little GHR 1A even at older ages (77 d). Despite the variation, a tendency for an effect of age ($P < .10$) on GHR 1A mRNA was detected because GHR 1A increased in older pigs (Figure 4C). An effect of age was not detected by ANOVA for exon 2–3 ($P = .16$), despite an apparent age-related increase that was detected with mean separation procedures. There was no effect of age on IGF-I mRNA.

The GHR 1B and exon 2–3 (within the GHR 1B assay) were expressed by 21 d of age (Figure 4B). There was no effect of rpST treatment or treatment × age interaction for the expression of GHR 1B or exon 2–3. There was no effect of age on GHR 1B mRNA (Figure 4C). There was a tendency for an effect of age ($P < .10$) on the exon 2–3 fragment in the GHR 1B assay because exon 2–3 increased with increasing age.

Experiment 3. The liver of pregnant gilts contained GHR 1A, IGF-I, and GHR 1B mRNA (Figure 5). Gilts treated with rpST tended to have decreased expression of liver GHR 1A ($P < .10$) and had increased liver IGF-I mRNA ($P < .01$) (Figure 5A and 5C). There was no effect of rpST on the expression of GHR 1B mRNA (Figure 5B and 5C).

Discussion

Animal growth is a complex process that involves an interaction of genetic, hormonal, and nutritional factors. Two of the most-studied hormones that control animal growth are GH and IGF-I (Stewart and Rotwein, 1996; Etherton and Bauman, 1998; Simmen et al., 1998). Growth hormone binds to the GHR, and the GHR transmits an intracellular signal through the JAK/STAT second messenger system (Carter-Su et al., 1996). A 4.2-kb GHR mRNA encodes the GHR protein in pigs (Louveau and Etherton, 1992; Wang et al., 1993). In other species, alternative splicing of the GHR mRNA leads to a heterogeneous pool of GHR mRNA with different exon 1. The different exon 1 sequences suggest that multiple promoters control GHR (Edens and Talamantes, 1998; Schwartzbauer and Menon, 1998). The two primary GHR promoters (GHR P1 and GHR P2) transcribe GHR 1A and GHR 1B mRNA, respectively (Schwartzbauer and Menon, 1998). A third promoter (GHR P3) has recently been discovered in cattle and transcribes GHR 1C mRNA (Jiang et al., 1999). Expression of GHR mRNA from different promoters may provide greater capacity for regulating GHR mRNA in response to metabolic or physiologic stimuli. For example, GHR 1A mRNA responded to hormonal signals, such as estradiol and GH (Baumbach and Bingham, 1995; Gabrielsson et al., 1995; Kobayashi et al., 1999), whereas GHR 1B mRNA remained unchanged. The GHR 1B may be a constitutive mRNA, and its expression may satisfy a basal requirement for cellular GHR. The GHR 1C mRNA is also constitutive, and its pattern of expression is similar to GHR 1B mRNA (Jiang et al., 1999). Pigs are similar to other species because we cloned two exon-1 GHR mRNA variants (GHR 1A and GHR 1B) from the pig liver. The presence of two GHR variants suggests that multiple GHR promoters exist in pigs. These two variants are homologous with GHR 1A and GHR 1B found in other species.

Ribonuclease protection assays were developed to investigate the expression of GHR 1A and GHR 1B mRNA in porcine tissues. Both GHR 1A and GHR 1B mRNA were expressed in pig liver. The GHR 1B mRNA but not the GHR 1A mRNA was detected in muscle (77-d-old pigs) as well as in uterus and ovary (pregnant pig). Therefore, pigs are like other species because GHR 1B was found in a variety of tissues, whereas GHR 1A was a liver-specific mRNA (O'Mahoney et al., 1994; Adams, 1995; Baumbach and Bingham, 1995; Pratt and Anthony, 1995; Southard et al., 1995; Lucy et al., 1998). We did not attempt to characterize the developmental regulation of GHR 1A or GHR 1B mRNA in tissues other than liver. Instead, the present study focused on liver expression of GHR 1A and GHR 1B mRNA. We cannot preclude the expression of GHR 1A mRNA in other tissues during other developmental periods or other physiological statuses. Although GHR 1A is generally considered a liver-specific mRNA (Edens and Talamantes, 1998; Schwartzbauer and Menon, 1998), the

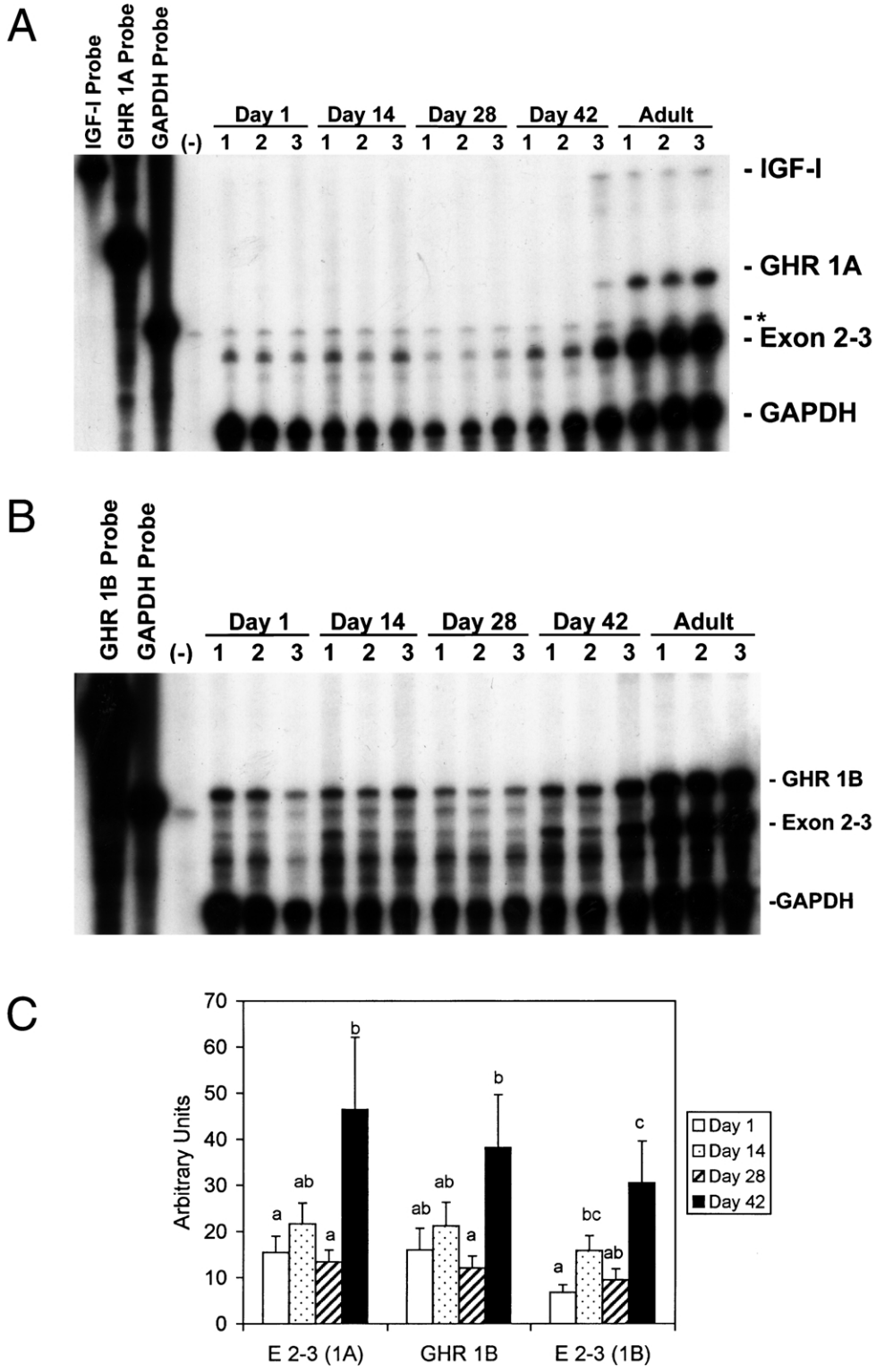

Figure 3. Autoradiograph of a ribonuclease protection assay (RPA) for GH receptor (GHR) 1A, IGF-I, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA (A); and GHR 1B and GAPDH mRNA (B); in liver of neonatal (1 d of age) and castrated (14, 28, and 42 d of age) male pigs. Three individual pigs (1, 2, and 3) are shown for each age. Both GHR 1A and IGF-I required long exposures to visualize the signal before 28 d (data not shown). Adult liver is from control pregnant pigs (Exp. 3). For each RPA, the locations of GHR 1A, GHR 1B, GHR exon 2–3, and GAPDH protected fragments are shown at right. Undigested probes as well as negative control (yeast RNA) (–) are also shown (left). The asterisk denotes residual of undigested GAPDH probe. (C) Means and standard error (bars) for the exon 2–3 signal from the GHR 1A assay [E 2–3 (1A)], GHR 1B, and exon 2–3 signal from the GHR 1B assay [E 2–3(1B)] from Exp. 1. Within a mRNA, bars lacking a common letter differ at $P < .05$.

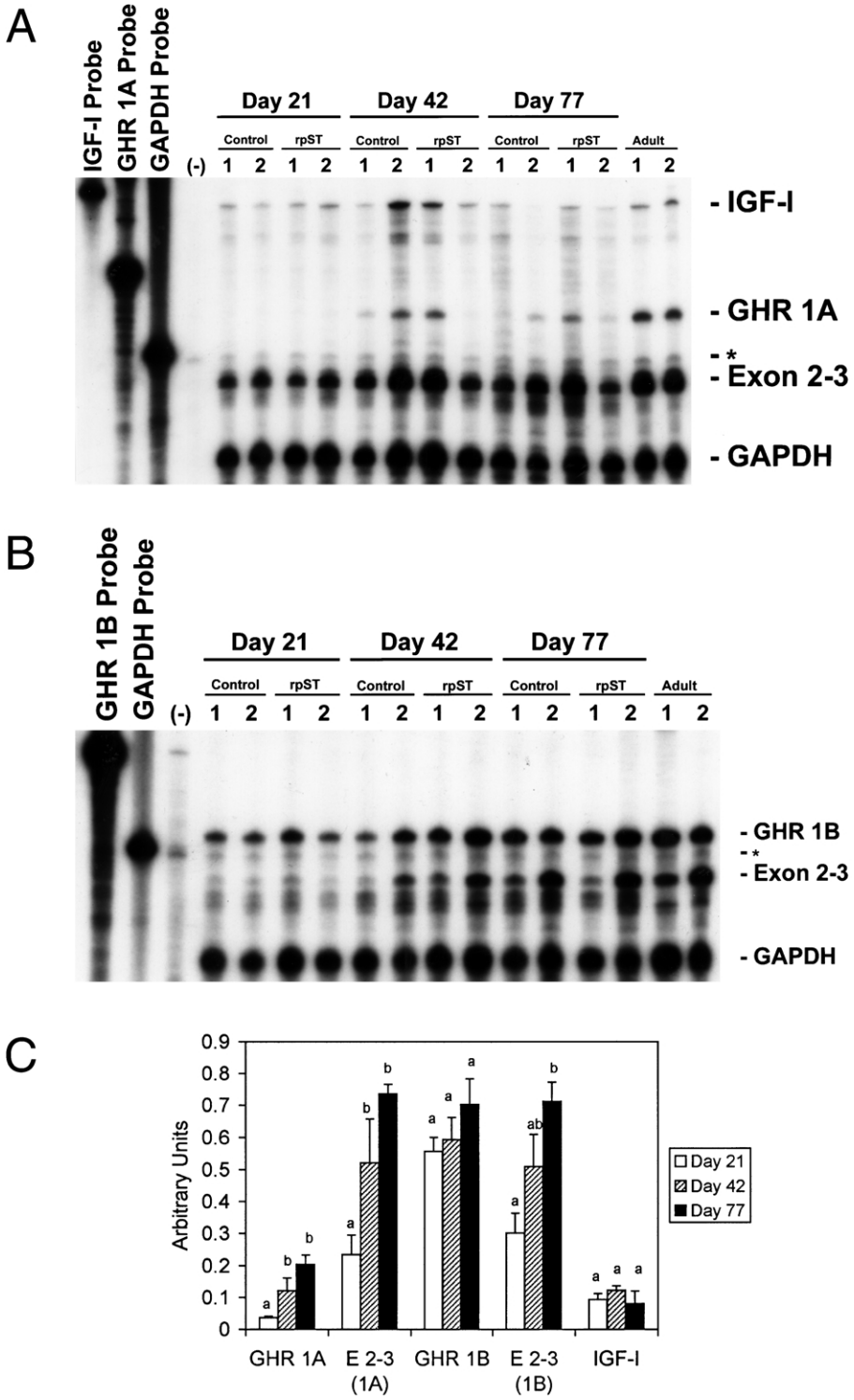

Figure 4. Autoradiograph of a ribonuclease protection assay (RPA) for GH receptor (GHR) 1A, IGF-I, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA (A); and GHR 1B and GAPDH mRNA (B); in liver of intact male pigs treated with or without recombinant porcine ST (rpST) for 14 d prior to slaughter at 21, 42, and 77 d of age. Two individual pigs (1 and 2) are shown for each treatment at each age. Adult liver is from control pregnant pigs (Exp. 3). For each RPA, the locations of GHR 1A, GHR 1B, GHR exon 2–3, and GAPDH protected fragments are shown at right. Undigested probes as well as negative control (yeast RNA) (–) are also shown (left). The asterisk denotes residual of undigested GAPDH probe. (C) Means and standard error (bars) for GHR 1A, exon 2–3 signal from the GHR 1A assay [E 2–3 (1A)], GHR 1B, exon 2–3 signal from the GHR 1B assay [E 2–3(1B)], and IGF-I from Exp. 2. The effects of rpST were not significant. Therefore, bars represent pooled means for rpST and control pigs. Within a mRNA, bars lacking a common letter differ at $P < .05$.

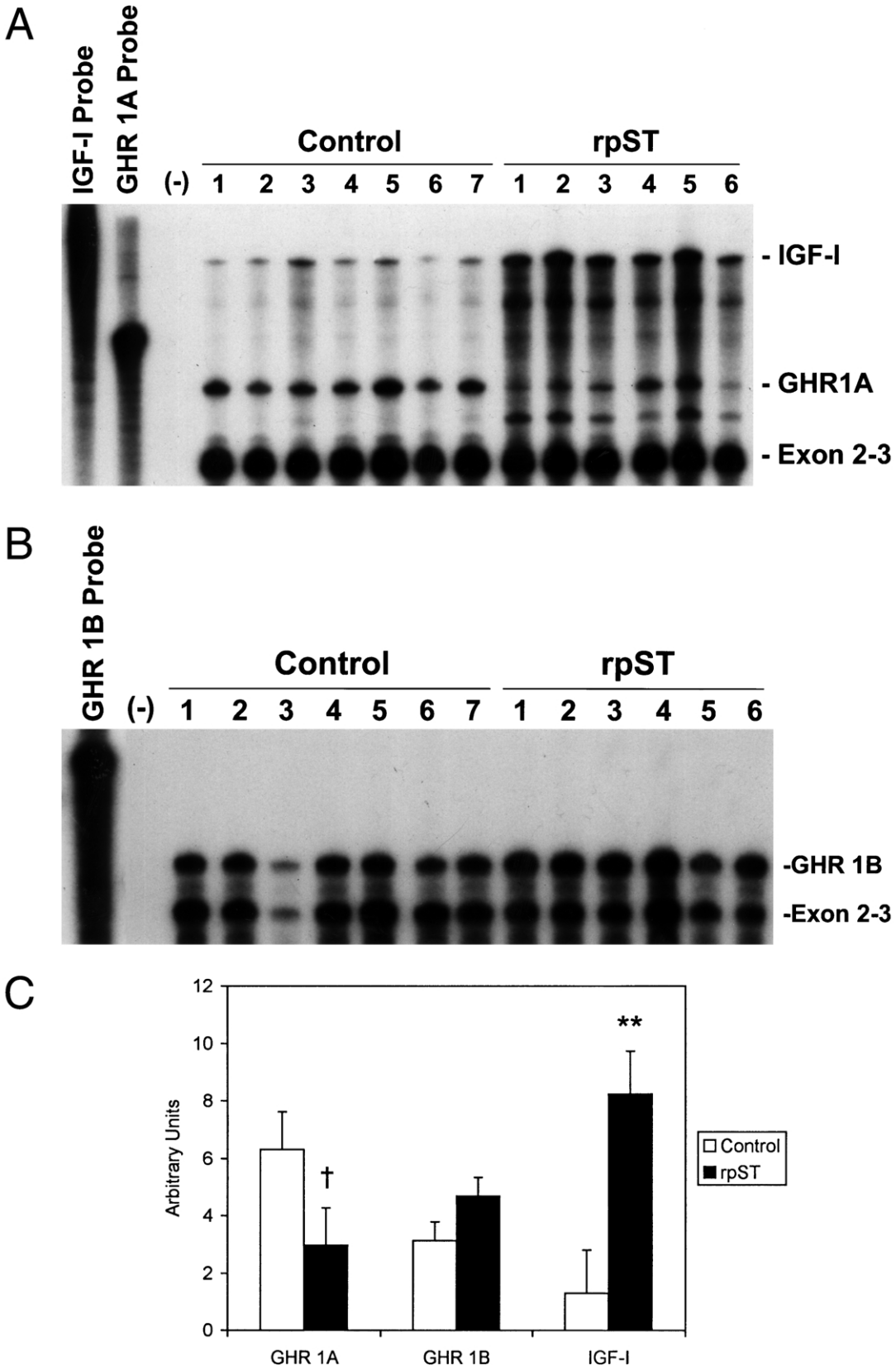

Figure 5. (A) Autoradiograph of a ribonuclease protection assay (RPA) for GH receptor (GHR) 1A and IGF-I mRNA (A); and GHR 1B mRNA (B); in pregnant gilts treated with saline (control; n = 7) or recombinant porcine ST (rpST) (n = 6). For each RPA, the locations of IGF-I, GHR 1A, GHR 1B, and exon 2–3 protected fragments are shown at right. Undigested probes as well as negative control (yeast RNA) (–) are shown (left). (C) Least squares means and standard error (bars) for GHR 1A, GHR 1B, and IGF-I signal from Exp. 3. †$P < .10$, **$P < .01$.

potential for expression outside of liver during unique physiological circumstances has not been explored. We have also not addressed the regulation of GHR in adipose tissue, where a high level of GHR 1B is found in other species (Lucy et al., 1998).

The expression of GHR mRNA increased with age. These data agree with previous studies of GHR mRNA that measured total GHR and found greater GHR mRNA in older animals (Peng et al., 1996; Schnoebelen-Combes et al., 1996). The IGF-I mRNA amount was near the level of assay detection for Exp. 1 (neonatal and castrated males) but could be detected in Exp. 2 (intact males). Although the IGF-I data arise from separate studies, they are consistent with lower blood IGF-I in castrated pigs (Louveau et al., 1991). The IGF-I mRNA was not like GHR mRNA because IGF-I mRNA did not increase with age. These data also agree with previous analyses for pigs that show little change in IGF-I mRNA in developing pig liver despite increases in GHR mRNA and blood IGF-I (Lee et al., 1993; Peng et al., 1996; Carroll et al., 1999). Therefore, the increase in blood IGF-I that occurs in growing pigs may not arise from liver IGF-I synthesis.

This seems to be the first study to measure the individual GHR variants in growing pigs. Expression of GHR 1A and GHR 1B mRNA in pigs increased with age. In castrated male pigs (Exp. 1), GHR 1A mRNA was poorly expressed until 42 d of age. However, GHR 1B mRNA was expressed earlier in life (1 d of age) and increased to a maximum at 42 d of age (last time point tested). In intact male pigs (Exp. 2), GHR 1A mRNA was also present by 42 d of age and increased to 77 d of age. The developmentally regulated expression of GHR 1A and GHR 1B mRNA has been reported for other species and seems to be an evolutionarily conserved mechanism for GHR regulation (Schwartzbauer and Menon, 1998). The timing of the initiation of transcription for each GHR mRNA, however, differs among species. In sheep, for example, GHR 1A mRNA was expressed in fetal liver during late gestation (Pratt and Anthony, 1995), and, in cattle, a large increase in GHR 1A mRNA occurred within 1 mo of age (Smith et al., 1998). Rats and mice differ from sheep or cattle because there is a steady increase in GHR 1A and GHR 1B mRNA during the early developmental period with an increase in GHR 1A after puberty in the female (approximately 4 wk of age) (Baumbach and Bingham, 1995). Although puberty increases GHR 1A in rat and mouse, pregnancy is the primary mechanism through which GHR 1A mRNA is increased. Pregnant pig liver was used as a positive control in these experiments because it had abundant GHR 1A and GHR 1B mRNA. The effects of porcine pregnancy on liver GHR, however, will need to be tested in controlled experiments.

With respect to developmental regulation, pigs are similar to rats because both GHR 1A and GHR 1B mRNA increased with age. In castrated pigs (Exp. 1), there was a depression in GHR (either GHR 1B or exon 2–3) expression on d 28. An increase in serum GH was also observed in the same pigs at 28 d of age (Matteri and Carroll, 1997). The increase in GH and the decrease in GHR mRNA may be associated with the developmental maturation of the hypothalamic-pituitary axis that occurs between 3 and 5 wk of age in pigs (Buonomo and Klindt, 1993). An increase in GHR 1A was present by 42 d of age in the castrated and intact male pigs (Exp. 1 and 2). The initiation of GHR 1A expression was variable, however, and some pigs did not have GHR 1A mRNA at the latest time point (77 d). The reason that GHR 1A mRNA expression was highly variable was not clear, but GHR 1A was not correlated with body weight at slaughter or weight gain preceding slaughter (data not shown). Therefore, other developmental mechanisms mediate the changes in GHR 1A that we observed.

We measured GHR 1A and GHR 1B mRNA because these GHR variants are found in a variety of species. We recently characterized a third GHR mRNA (GHR 1C mRNA) in cattle. Additional GHR variants are found in humans (eight known variants) (Pekhletsky et al., 1992) and rats (five known variants) (Domene et al., 1995). The exon 2–3 fragment in the GHR RPA represents GHR variants that do not contain the exon 1 of the probe. In the GHR 1B assay, the exon 2–3 fragment represents GHR 1A and any other GHR mRNA variants. In general, the exon 2–3 signal within one GHR RPA reflected changes that were seen in the alternative RPA. For example, the increase in the exon 2–3 signal within the GHR 1B assay in Exp. 2 was associated with an increase in the GHR 1A signal. This suggests that GHR 1A and GHR 1B are major components of GHR mRNA in pigs. The association did not always hold true, however, because in Exp. 1 we observed an exon 2–3 signal in the GHR 1B assay before d 28 when we detected very little GHR 1A mRNA. Therefore, there may be additional GHR mRNA variants that contribute to the GHR pool of growing pigs. The presence of multiple GHR variants controlled by multiple promoters needs to be explored at the molecular level.

In rats, the GHR 1A mRNA was not increased by injections of GH, but continuous infusion of GH caused an increase in GHR 1A mRNA (Baumbach and Bingham, 1995). In Exp. 2, there was no effect of rpST injection on either GHR 1A or GHR 1B mRNA. The different responses in pigs and rats may be related to method of administration (injection vs infusion) or unique aspects of GHR regulation by GH in rats vs pigs. In pigs, concentrations of GH were declining during the period that GHR 1A expression was initiated (Dubreuil et al., 1987; Louveau et al., 1991). Therefore, it is unlikely that GH exerts a positive feedback effect on the GHR in growing pigs. Patterns of GH secretion are different in rats, however, and concentrations of GH increase with age (20 to 90 d) and assume a male (high amplitude, low baseline) vs female (low amplitude, high baseline) pattern of expression around puberty (Eden, 1979). The timing of GHR 1A expression seemed to coincide with the maturation of the GH/IGF-I system, but the system

was not fully functional because the pigs in Exp. 2 did not demonstrate an increase in IGF-I mRNA in response to rpST. Therefore, GH insensitivity in young pigs (lack of IGF-I response) was associated with the failure of GH-induced induction of GHR 1A mRNA. There was a tendency for decreased in GHR 1A in pregnant pigs treated with rpST (Exp. 3). It will be necessary, however, to examine the effects of GH on GHR 1A in growing pigs that are rpST-responsive without the complicating effects of pregnancy.

The general trend that we observed was for an increase in GHR mRNA with age regardless of the type of GHR mRNA that we measured (either GHR 1B [28 to 42 d of age] or GHR 1A [21 to 42 d of age]. This is distinctly different from sheep and cattle, in which a large increase in GHR 1A occurs independently of other GHR variants (Pratt and Anthony, 1995; Smith et al., 1998). The GHR 1A and GHR 1B mRNA, however, were not always controlled in a coordinated manner. When we examined GHR 1A and GHR 1B in pregnant pigs, we found that GHR 1A tended to be decreased in response to rpST, but GHR 1B remained unchanged. The responses that we observed were typical of GHR 1A and GHR 1B in rats and cattle because GHR 1A responded to a metabolic signal (rpST), whereas GHR 1B remained unchanged (Baumbach and Bingham, 1995; Kobayashi et al., 1999). In most species, GHR 1A increases in response to GH. The surprising response in pregnant pigs was that rpST tended to decrease GHR 1A mRNA. The endocrine and metabolic mechanisms that cause the coincident increase in IGF-I and decrease in GHR 1A are completely unknown.

In conclusion, at least two variants of GHR mRNA (GHR 1A and GHR 1B) exist in pigs. The expression of each was tissue-specific and developmentally regulated. The GHR 1A was a liver-specific GHR mRNA that increased at 42 d of age. The GHR 1B mRNA was detected earlier in life in liver as well as other tissues, including muscle, uterus, and ovary. Recombinant pST had no effect on GHR 1A or GHR 1B before 77 d of age but tended to cause a decrease in GHR 1A in the pregnant pig. Total GHR mRNA in pigs, therefore, consists of multiple GHR variants that are uniquely regulated through developmental, tissue-specific, and metabolic mechanisms.

Implications

Growth in pigs depends, at least partially, on growth hormone and the growth hormone receptor. The pig liver seems to express at least two variants of growth hormone receptor. One variant (1A) seems to be limited to liver, whereas a second variant (1B) seems to be in a variety of tissues. The growth hormone receptor 1B seems to be present at younger ages than the growth hormone receptor 1A. Therefore, growth hormone receptor 1B is the predominant form of the growth hormone receptor in pigs before 77 d of age. The growth hormone receptor 1A can be detected later in life, and its specific function relative to 1B is not known. Future studies should identify the independent mechanisms that control growth hormone receptor 1A and 1B in pigs.

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